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THE MICROBIOLOGY OF THE UPPER AIR. I.

By Bernard E. Proctor.

(Continued from page 3 of cover.)

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By Bernard E. Proctor.

Read April 11, 1934. Introduced by J. W. M. Bunker.

The collection of micro-organisms from the air at various altitudes has interested observers for a considerable time. Pasteur, one of the first workers in this field, made investigations by climbing the Alps to obtain his samples. More recently the airplane has made it possible for others to make studies on the micro-organisms found in the upper air which occur in various regions.

Stakman, Henry, Curran, and Christopher found numerous spores in airplane flights in Texas, Minnesota, Nebraska, and Wyoming.[1] Mischustin reported results obtained over the Moscow experimental flying field.[2] Craigie and Popp have used airplanes in collecting rust spores from the upper air in Canada.[3] Brown made collections of living micro-organisms in the air of the arid section in southwestern United States.[4] Meier, Stevenson and Charles have identified fungus spores collected on flights over eastern United States in 1932.[5]

The establishment in 1931 of a daily airplane flight service by the Division of Meteorology of the Department of Aeronautical Engineering at the Massachusetts Institute of Technology suggested an opportunity to study the microbiology of the upper strata in the vicinity of Boston. The first flight on which studies of a biological nature were undertaken was on February 23, 1932. Through the courtesy and coöperation of the staff of the Division of Meteorology it has been possible to collect a considerable number of biological samples which were immediately taken to the laboratories of the Department of Biology and Public Health for biological examination.

Preliminary Experiments.

On the first flight a series of six sterilized Petri plates containing standard nutrient agar was used for the collections at different altitudes. Each of these plates was exposed separately by opening it

[1] Stakman, E. C., Henry, A. W., Curren, G. C., and Christopher, W. N. Jour. Agri. Research. **24** (7): 599–606, 2 pls. 1923.

[2] Mischustin, E. Centralblatt f. Bakt. etc., II. Abt., Bd. **67**: 347–351. 1926.

[3] Craigie, J. H. and Popp, W. Rust Epidemiology. Canada, Dept. Agric., Exper. Farms, Report of the Dominion Botanist for 1927: p. 47–54. 1928.

[4] Brown, J. G. Science **72**: 322–323. 1930.

[5] Meier, F. C., Stevenson, J. A., and Charles, V. K. Phytopathology **23**: 23. 1933.

just outside of the cabin. The lower half of the plate was extended at arm's length from the cabin door and exposed to the air for one minute while the plane was operated at an approximate speed of 100 miles per hour. At the end of this time, the cover was replaced and the closed plate returned to the cabin. The plates were immediately returned to the laboratory and incubated for 72 hours at 20° C. Table I contains the data obtained from this flight, together with the temperatures at each exposure altitude.

TABLE I.

MICRO-ORGANISMS OBTAINED ON FLIGHT A.

Petri plate	Altitude ft.	C° Temp.	Mold count	Bacteria count
1	13,300	−35	60	23
2	10,700	−28	10	31
3	7,600	−23.5	15	36
4	5,800	−18	14	20
5	5,700	−18	18	20
6	2,000	−8	50	25

A short time later four more flights were made, each on a separate day, during which collections were taken. The Petri plates were exposed for one minute each at different elevations.

Previous to the day of exposure, all the Petri plates used in these experiments were observed in the laboratory for 2 days to make sure that no contaminating organisms were present. Surgeon's tape was used to seal the upper and lower sections of the plates, between the airport at East Boston and the laboratory, and the plates were further protected during transportation by being placed in a tight, sterile, metal box.

Precautions were also taken to exclude dust contamination in the cabin by opening the plates for exposure only after thrusting them out the door. The results obtained are included in Table II.

TABLE II.

MICRO-ORGANISMS OBTAINED ON FLIGHTS B, C, D AND E.

Altitude	Flight B		Flight C		Flight D		Flight E	
in ft.	Bact.	Molds	Bact.	Molds	Bact.	Molds	Bact.	Molds
1,500	218	300	—	—	16	21	—	—
3,000	179	5	11	0	27	8	137	5
6,000	4	2	33	43	3	5	8	1
9,000	4	0	5	3	1	0	191	1
12,000	12	0	5	0	1	98	14	3
15,000	8	0	4	1	7	0	39	6

The preliminary observations indicated the need of a type of apparatus which would enable micro-organisms to be collected from the upper air with no danger of contamination from other sources. No known device was found to meet the definite limitations of weight, size, wide temperature range, and need for semi-automatic operation which were imposed by the fact that the flights of this plane were primarily concerned with meteorological research and the single occupant served the dual rôle of pilot and observer. In addition it was hoped that examinations could be made simultaneously for the presence of pollen, and possibly for dust, in the air.

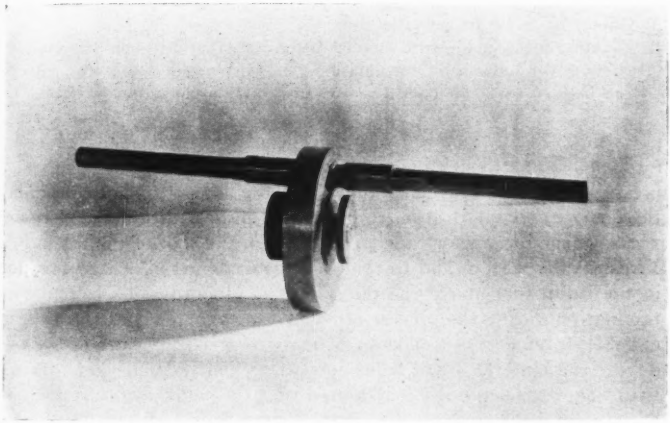
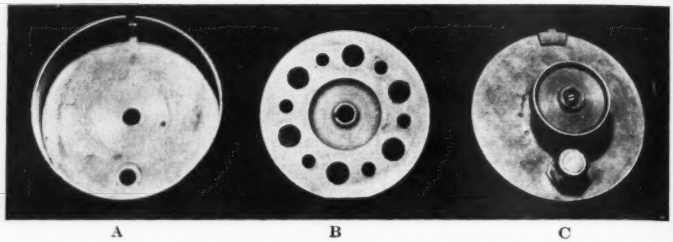


FIGURE 1. Collecting apparatus assembled. Tubes for air inlet and outlet connected.

After numerous tests with a variety of devices, a collecting apparatus was devised which, though not ideal, met most of the limitations mentioned. In essence, this apparatus, Fig. 1, was a cylindrical brass compartment five inches in diameter and less than an inch in depth, within which was a rotating metal plate, Fig. 2. When this plate was rotated, it was possible to expose separate disks of especially treated lens paper in such a manner that an air current drawn through the compartment passed through the interstices of the paper which acted as a filter.

The filter disks were first washed in chloroform, then dried and treated with a highly purified hydrocarbon oil having a congealing



A B C

FIGURE 2. Parts of collecting apparatus.

A. Outer shell, showing air outlet.
B. Rotating metal plate, with circular wire screens used to hold paper disks.
C. Front cover. Knob for rotating inner metal plate in the center. Metal cap used to cover air inlet during transportation shown below knob.

point of − 71° C., before being placed in the apparatus. The entire mechanism was then subjected to heat sterilization with steam at a pressure of 20 pounds for 30 minutes in the laboratory. On reaching the airport at East Boston, the inlet and outlet to the compartment were attached to a short line going through the roof of the plane to a Venturi tube, Fig. 3, and to the collector attached in a fixed position in the cabin just above the pilot, Fig. 4. In order to obviate contamination during the process of connecting the apparatus, a free air channel through the collector is maintained by means of a hole drilled through the inner rotating plate which may be kept in line with the air stream until it is desired that the collection start. There are six paper filters for collecting micro-organisms in the apparatus and six free holes between, making it possible to air-wash the entire system after each exposure. A one minute period of air-flow through the free holes was observed as a regular procedure between exposures.

Certain control measures were instituted to insure, so far as possible, that such bacteria as were obtained came from the air at the levels indicated. The results of a flight were rejected if the control disk, always left unexposed, was not found sterile on subsequent examination.

One flight, during which an exposure was made while the plane taxied along the ground, showed that subsequent exposures might be sterile, although one exposure contained many micro-organisms. This tends to indicate that if the air flow through the open hole intervened between exposures, there was but slight possibility of contamination at higher altitudes.

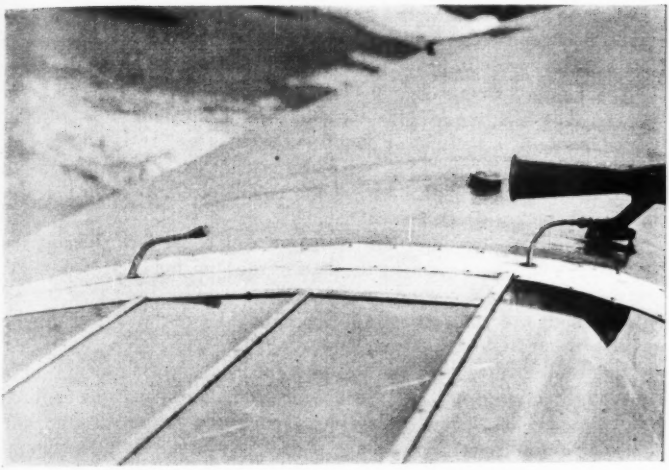


FIGURE 3. Roof of plane, showing Venturi tube at right and air inlet tube on left.

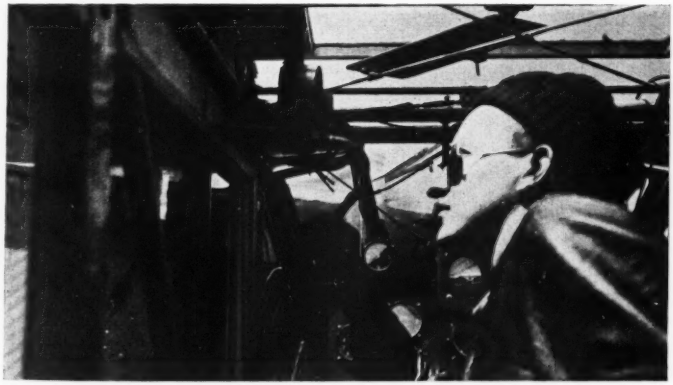


FIGURE 4. Interior of cabin of plane, with collection apparatus installed. (Pilot, Lt. H. B. Harris).

No claim is made that this collecting device arrests all the microorganisms from the air which passes through it. The use of a water trap in series with it proved definitely that such was not the case. If indeed such a device were a perfect trap, the air flow would be so reduced that it would be questionable whether any organisms would be obtained in a reasonable length of exposure. It is considered, however, that the apparatus has many advantages over Petri dishes as a collecting agent and affords some means of comparison between various exposures.

Tests conducted to determine the approximate air flow through the collecting device were made by the staff of the Division of Meteorology, using the Technology wind-tunnel. These results indicated an air flow through the collector of approximately 1 cubic foot per minute under general flight conditions.

The usual flights were slow, more or less steady ascents in a westerly direction from East Boston until the highest altitude (ceiling) was reached that seemed advisable, then a more rapid descent and return to the airport. This plane, Fig. 5, a Curtiss-Robin equipped with a Challenger 185 H. P. engine, when carrying all its numerous meteorological instruments reaches, under optimum conditions of flight, a height of about 20,000 feet. Such an altitude was not commonly attained in most of the flights under discussion, 17,000 to 18,000 feet being the usual height reached.

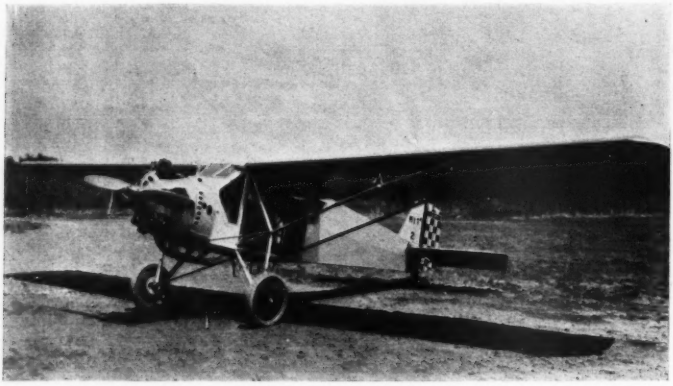


FIGURE 5. Airplane used in making collections, through the coöperation of the Division of Meteorology, Massachusetts Institute of Technology.

Laboratory Procedure.

The collection apparatus was returned to the laboratory on the completion of each flight. If the following examinations could not be carried out within a few hours, the "collector" was kept in a mechanical refrigerator until the next day.

Using aseptic technique, the oiled paper filters were removed from the collector and placed in a specially sterilized glass cell, covered by a large microscope cover slip. Each paper disk was then examined with a microscope, using a 16 mm. objective and a 10× ocular lens. By means of a mechanical stage it was possible to observe and sketch any pollen grains which were found, and in a number of the flights an examination and count of dust particles was made simultaneously.

On completion of direct microscopic examination, the paper disks were removed from the glass cell with sterile forceps and each placed in a separate tube containing 10 cc. of sterile water. After a period of five minutes' soaking, the tubes, plugged with sterilized rubber stoppers, were shaken thoroughly until the paper disks disintegrated into separate fibers. Then the water in each tube was transferred with sterile pipettes to sterile Petri dishes, using two or three cc. to each plate. Immediately thereafter standard nutrient agar was added in the usual manner, allowed to cool, and the plates incubated at 20° C. for 5 days. At the end of this time they were placed in a 37° C. incubator for 2 days. Counts were made after each incubation period. Under the above conditions no anaerobic organisms would be capable of growth.

Bacteria.

Table III gives the results obtained from exposures made on 40 flights. No attempt was made to determine the number of organisms according to length of exposure. As the exposures were made over a considerable range of altitude, at each level of which the air masses are likely to vary considerably, a ratio of micro-organisms to time would likely be of little value. It would have been ideal if the exposures could have been made at definite fixed altitudes for certain periods of time. This was possible only in one flight, because the primary purpose of the flights was for meteorological observations, which necessitate different procedure.

As the temperatures at the greatest elevation and at the ground are included, it will be noted that the temperatures are rather low, particularly during the first of this series, which started in January, 1933.

TABLE III.

BACTERIA AND MOLDS AT DIFFERENT ALTITUDES.

Flight No.	Altitude 1000 ft.	Minutes exposed	Bact.	Molds	Temp. °C.
1	1.0–7.0	10	4	9	G. 2.6
	9.–14.0	10	5	0	
	14.4–16.6	10	2	0	
	16.8–17.8–14.2	10	1	2	−21.5
	11.6–.5	10	18	1	
2	15.5–17.5	10	3	2	−26.5
	17.5–11.5	10	1	2	
	9.5– .5	10	28	22	G. 4.5
3	12.5–16.8		0	71	
	17.0–19.1–18.0		1	73	−22.5
	17.8–gr.		18	108	G. 1.0
4	1.0–7.6	10	0	0	
	9.0–14.0	10	4	1	
	14.4–16.5	10	0	2	
	16.5–17.2–14.0	10	0	0	−31.5
	11.5–gr.	10	2	3	G. 2.8
5	1.0–7.5	10	0	0	
	9.0–14.0	10	0	0	
	14.4–16.6	10	1	0	
	16.8–17.7–14.0	10	1	1	−22.
	9.5–gr.	10	3	12	G. − 2.
6	1.4–9.1	12	1	0	
	9.8–13.5	12	0	5	
	14.5–15.2	12	0	0	
	15.4–18.0	12	0	0	−18.
	18.4–gr.	12	18	7	G. − 2.
7	1.0–9.0	12	0	1	G. − 1.5
	9.4–13.4	12	2	0	
	13.6–14.6	12	1	1	
	15.4–17.4	12	0	1	
	18.0–11.4	12	2	2	−28.

TABLE III—cont.

Flight No.	Altitude 1000 ft.	Minutes exposed	Bact.	Molds	Temp. °C.
8	2.0–10.0	12	2	0	
	11.0–14.0	12	0	0	
	14.5–16.8	12	0	1	
	17.0–18.2	12	0	0	−22.
	16.0–gr.	12	9	11	G. 0
9	3.0–9.5	12	0	0	
	10.5–13.5	12	1	2	
	13.8–15.4	12	1	0	
	17.2–18.2	12	0	0	−33.
	13.5–gr.	12	0	1	G. 3
10	3.0–9.5	12	1	0	
	10.5–13.5	12	0	0	
	13.8–15.4	12	1	0	
	16.0–17.2	12	0	0	−30.
	12.5–gr.	12	9	2	G. −12.
11	2.0–9.0	12	3	1	G. − 2.
	10.0–15.8	20	3	9	
	16.4–17.6	12	1	0	−17.
12	7.0–11.2		2	1	G. 13
	12.4–14.2		2	0	
	14.4–15.5		0	0	
	16.0–17.0–5.0		0	0	—13
	4.2–2.6		0	11	
13	7.4–16.2	20	0	0	
	16.4–18.8–10.0	20	3	0	−22.
	9.0–gr.	20	18	0	G. −12
14	3.0–14.6	23	0	0	
	14.8–17.5	20	0	0	
	17.6–18.8	20	0	0	−32
	17.5–gr.	20	28	0	G. − 6
15	gr. –11.2	17	0	0	
	11.4–16.1	17	0	0	
	16.2–17.2	17	0	0	
	17.2–17.4–5.0	17	0	0	−27
	5.0–gr.	6	10	43	G. 2

TABLE III—cont.

Flight No.	Altitude 1000 ft.	Minutes exposed	Bact.	Molds	Temp. °C.
16	gr. –10.0	15	1	1	
	10.6–14.8	10	0	0	−39
	14.4–gr.	20	0	0	G. 4
17	1.0–9.2	15	1	1	
	11.3–15.0	15	0	0	G. 9
	15.4–16.2	15	0	0	
	16.2–16.4–1.0	15	0	1	−30
18	5.0–10.7	15	0	0	
	11.0–13.4	15	2	1	
	13.6–18.0	15	1	0	
	18.2–18.8	15	0	0	−34
	17.0–gr.	15	2	0	G. − 4
19	.5–9.0	20	2	0	G. 1
	9.4–12.8	20	0	0	
	13.4–17.0	20	0	0	
	17.5–18.6	20	0	0	−32
	18.2– .5	20	3	0	
20	1.2–10.6	15	2	1	G. 0
	10.8–14.5	15	1	0	
	14.7–16.9	15	0	0	
	17.2–18.0–7.5	15	0	0	−29
21	1.0–12.0	15	0	0	G. −11
	12.2–16.0	19	0	0	
	16.0–17.2	15	6	0(1Y)	
	17.2–19.6	15	0	0	
	19.6–20.6	15	4	2	−36
22	12.8–16.0	17	0	0	
	17.5–18.8	17	0	0	−27
	17.8–gr.	17	11	0	G. − 4
23	1.0–10.6	15	0	0	G. 3
	10.8–15.4	15	1	0	
	15.8–17.8	15	0	0	
	18.0–18.4–11.6	15	0	0	−23
	11.4–5.0	15	1	1	

TABLE III—cont.

Flight No.	Altitude 1000 ft.	Minutes exposed	Bact.	Molds	Temp. °C.
24	gr. –10.0	15	4	0	
	11.4–14.8	15	0	0	
	14.9–16.4	15	0	0	
	16.6–18.2	15	0	0	−25
	18.2–gr.	15	0	0	G. 4
25	1.0–10.6	15	0	0	
	11.0–15.2	15	0	0	
	15.4–18.0	15	1	0	
	18.2–19.4–15.0	15	0	0	−38
	13.0–gr.	15	5	0	G. 3
26	2.0–11.0	20	0	0	G. 5
	11.8–17.0	20	0	0	
	17.2–19.4	20	1	0	−31
27	1.5–8.6	20	8	0	
	7.2–13.0	20	0	0	G. 9
	13.4–15.4	20	4	0	
	15.6–17.4	20	6	0	−10
	17.5–16.5	20	3	3	
28	1.0–10.0	20	0	1	
	11.2–15.4	20	1	0	G. 13
	15.7–18.4	20	0	0	
	18.5–19.8	20	0	0	−17
	19.8– .4	20	0	0	
29	1.0–10.0	15	0	0	G. 9
	11.5–16.5	18	0	0	
	16.8–18.3	15	4	0	
	18.4–19.6	15	0	0	−20
	19.6–1.0	15	0	0	
30	8.5–12.5	12	0	0	
	13.0–14.8	12	1	0	G. 2
	15.0–16.4	12	0	0	
	17.0–14.0	12	3	1	−25
31	.5–9.4	17	12	10	
	9.7–14.4	17	2	0	G. 14
	14.8–15.0	17	4	1	
	18.0– .5	17	13	13	−21

TABLE III—cont.

Flight No.	Altitude 1000 ft.	Minutes exposed	Bact.	Molds	Temp. °C.
32	1.0–12.5	19	1	0	
	12.9–16.2	19	1	0	G. 7
	16.4–18.6	19	0	0	−25
	18.8–19.2–2.6	19	0	0	
33	2.0–9.0	20	1	1(1Y)	G. 17
	9.4–12.2	20	0	0	
	13.4–15.4	20	0	0	−11
34	.5–10.0	15	0	1	
	10.4–14.4	15	0	2	G. 10
	15.1–17.3	15	0	0	
	17.3–18.2	15	0	0	−19
	17.0–gr.	15	4	0	
35	5.0–8.0	15	5	0	
	9.0–12.4	12	0	0	
	13.8–15.8	12	0	0	G. 14
	16.0–17.2	12	0	0	
	17.6–16.6	18	3	0	−16
36	2.0–	33	42	2	
	2.0–	33	28	2	
	1.0–	33	3	1	
	.5–	33	9	6	
	.5	33	6	4	G. 23.8
37	.5–7.6	15	2	1	G. 17
	7.8–12.4	15	1	2	
	13.0–15.4	15	0	3	− 1
38	.4–9.8	20	1	0	
	10.0–13.2	20	1	0	
	13.7–15.6	20	1	0	
	15.8–16.4–8.0	20	0	0	
	7.6–1.5	20	1	0	
39	gr. –9.0	15	3	0	
	9.1–14.1	15	0	0	
	14.2–17.2	15	4	0	
	17.3–18.4	15	2	0	

TABLE III—cont.

Flight No.	Altitude 1000 ft.	Minutes exposed	Bact.	Molds	Temp. °C.
40	gr. –4.0	13	0	0	
	9.5–13.8	13	0	0	
	14.0–15.8	13	1	0	

G.—Temperature at ground.
Y—Yeast cells.
gr.—Approaching ground—exact distance not recorded.

On numerous exposures bacteria were obtained when molds were absent. The converse is sometimes true also. No general rule can be made to cover all observations, but no entire flight was made in which all exposures were sterile.

In some flights somewhat larger counts for bacteria have been obtained at the lower levels, but this was reversed in flights on other days. One notable exception is Flight 21, where the lower exposures show no micro-organisms, then during an exposure from 16,000 to 17,200 feet some bacteria and a yeast cell were obtained. After another sterile exposure, the highest level attained in these flights (19,600–20,600 feet) showed the presence of bacteria and molds. Somewhat similar findings in some other flights would seem to indicate the possibility of definite strata, in which the micro-organisms may be present in somewhat higher concentration, due to conditions of temperature, humidity, and air movement which are of great importance in the formation and stratification of air masses. Precipitation must also play an important role in the distribution of bacteria.

The results obtained are not strictly comparable to those obtained by others noted in the literature, as the methods used previously have been decidedly different. They present the possibility of comparison with one statement of Meier, Stevenson, and Charles, to the effect that there is a decided thinning out of fungi above 8,000 feet. As a number of the exposures were made under 9,000 feet, exposures made above and below that height were tabulated and compared. These results are summarized together with those of another group, which contains the counts only from exposures at elevations above 15,000 feet.

TABLE IV.

COMPARISON OF RESULTS OBTAINED FROM EXPOSURES AT VARIOUS ALTITUDES.

Total Exposures, each over 10 minutes	173
Exposures in which living micro-organisms were found	100
Exposures made at altitudes lower than 9,000 feet	74
Exposures in which living micro-organisms were found	50 (67.6%)
Exposures made at altitudes above 9,000 feet	99
Exposures in which living micro-organisms were found	50 (50.5%)
All exposures made at altitudes above 15,000 feet	34
Exposures in which micro-organisms were found	12 (35.3%)

These summarized results should be interpreted as only exhibiting trends, however, because numerous individual exposures in each group were found to be sterile. The trend, as indicated by these comparisons, is for a decrease at higher levels in the percentage of exposures showing micro-organisms. This may be an apparent rather than a true decrease, as the time factor has not been considered.

It may be stated that one is likely to find bacteria at any level up to and including the highest altitude reached in this study, namely, about 20,000 feet.

Flight 36, which was conducted primarily for the collection of gypsy moth larvae in connection with the Bureau of Entomology of the U. S. Department of Agriculture, offered an opportunity of making a series of long collections at definite altitudes. A report on the entomological aspects of these flights is presented elsewhere by Collins and Baker.[6] The exposures made for bacteria and molds on this flight showed higher counts at 2,000 feet than at either 1,000 feet or 500 feet. These findings suggest the presence of larger numbers of micro-organisms in the higher air strata, under certain atmospheric conditions, than may be present in somewhat lower strata at the same time.

Types of Bacteria Isolated.

A group of 295 bacterial cultures were picked from typical colonies obtained from upper air exposures. After the common dilution-plate methods, these cultures were stained and their morphology determined.

[6] Collins, C. W. and Baker, W. L. Exploring the Upper Air for Wind-borne Gypsy Moth Larvae. Jour. Econ. Entomology, **27**, (2): 320–327. 1934.

Of these, 154, or 52.2% were spore-forming bacteria or bacilli. Non-spore-forming rods constituted 20%, or 59 organisms in the group, and cocci made up the remaining 82 cultures, or 27.8%.

More than half of the bacteria were spore-formers, which are notably more resistant to drying, cold, and other adverse conditions, including in some instances at least, exposure to sunlight.

Gram stains have been made of 204 of the above organisms, with the following results:

	Gram +	Gram −	Total
Bacilli	82	27	109
Non-spore-formers	37	4	41
Cocci	50	4	54

From this group, 49 cultures of bacteria have been positively identified, according to the characteristics outlined in Bergey's Manual of Determinative Bacteriology, 4th edition, 1934.

They were classified into families and species according to the morphological, physiological, and biochemical characteristics usually determined according to the standard methods of the Society of American Bacteriologists.

The cultures were found to include 29 species of bacteria, which are shown in Table V, together with the number of times each species was identified.

TABLE V.

SPECIES OF BACTERIA IDENTIFIED FROM AIR CULTURES AND NUMBER OF TIMES EACH WAS IDENTIFIED.

Family—Bacillaceae		*Family*—Coccaceae	
Bacillus albus	1	Achromobacter tiogense	1
B. aterrimus	1	Flavobacterium breve	1
B. cereus	5	F. flavescens	1
B. freudenreichii	1	F. solare	1
B. globigii	1		
B. graveolens	3	Micrococcus candidus	1
B. luteus	2	M. conglomeratus	2
B. mesentericus	2	M. ochraceus	3
B. mycoides	1	M. percitreus	2
B. nondiastaticus	1	M. perflavus	1
B. platychoma	2	M. sensibilis	1
B. silvaticus	3	M. sphaeroides	1
B. simplex	1	Rhondococcus corallinus	1
B. subtilis	6	Sarcina citrea	1
Achromobacter geniculatum	1	S. flava	1

Of the total cultures of bacteria classified and identified, 30, or more than 50% are members of the Family Bacillaceae, which are the resistant spore-formers.

The most common species found were *B. cereus* and *B. subtilis*, both of which were isolated and identified in six different cases. Every species identified was found to be one which is commonly found in the soil, water, or air.

It is interesting to note that no pathogens were found. Neither *Escherichia coli* (*B. coli*) or similar intestinal types were found.

In the 155 bacteria which have not yet been identified, although their characteristics have been determined, certain chemical characteristics are interesting. Practically none of these cultures has the ability to ferment the common sugars with the formation of gas. Another feature that many have in common is the inability to produce indol. In many respects they resemble various species which are listed above, but differ in some of the characteristics.

Molds.

The data in Table III indicate the presence of molds in the air to a considerable height. Their occurrence seems subject to considerable variation and the numbers collected, using the methods outlined, are low. The largest counts are shown in Flight 3, where all the exposures had over 70 molds. A considerable number of flights collected no molds. It seems likely that the molds in the air are rather irregularly distributed, as some flights show molds to be present at high altitudes, (19,600 feet in Flight 21) and absent near the ground. In some instances they seem to be present at all altitudes, and in some other flights more numerous at lower altitudes, as in Flight 31. No attempt was made to draw quantitative conclusions, but there are indications that altitude is not a factor of great significance in respect to mold distribution. That molds can be lifted from the earth to considerable distances, as shown in Flight 21, may well be significant to those concerned with the spread of plant diseases caused by fungi.

It must be emphasized that only those types of molds which grow readily in nutrient agar would be expected in these findings. As it was necessary to grow the bacteria on the same media, it was essential that nutrient agar be used.

Types of Molds Isolated.

Typical colonies of molds obtained in the Petri dishes were subsequently transferred with aseptic precautions to test tubes. These

cultures were later plated in dilutions to assure relatively pure cultures, which were subsequently examined microscopically.

Examination of 128 mold cultures which were from typical colonies found on the Petri dishes, gave evidence that 57 of the molds were Aspergilli and 36 of the remainder were Penicillia.

Of the remainder, molds of the following genera have been partially identified: Alternaria, Cladosporium, Helminthosporium, Monilia, Trichoderma. Others await identification.

Yeasts.

A number of colonies which developed on the Petri dishes from the air collections resembled yeast colonies in appearance. Each of these was later transferred to malt extract broth and incubated for 48 hours at 30° C. At the end of this incubation period, a hanging drop culture was made and the preparation examined microscopically to determine whether the cells reproduced by budding.

Of those cultures examined, 18 resembled yeast from a morphological standpoint, but none of these cultures was capable of fermenting either sucrose or dextrose with the formation of gas.

Four other cultures were isolated in this group which resembled yeast in size but formed a filamentous branching mycelium. One of these, very dark in color, appeared to have characteristics similar to *Torula niger*.

Actinomycetes.

Several cultures were found to be members of that group intermediate between bacteria and molds, the Actinomycetes. No efforts have been made to carry these identifications further. These organisms are usually soil organisms.

Comparison of Biological Data with Atmospheric Conditions.

Table VI is a compilation of the total numbers of micro-organisms, together with such factors as temperature, weather observations at the time of flight, precipitation on day of flight and the day previous, wind direction and velocities at different altitudes. At the left of the table is the collection time, which varies in different flights to a considerable extent.

It can readily be seen that the biological results obtained on each flight are subject to a number of widely fluctuating variables. Comparisons are difficult under such circumstances.

In general, more bacteria were obtained on any specific flight than

molds, although Flight 3 is a notable exception. No flights were made which did not show bacteria in at least one exposure. Thirteen flights were made during which no molds were collected.

There is possibly a slight indication of lower micro-organism counts on flights made on days following precipitation. With such small numbers of micro-organisms, however, any change in counts within the range shown would appear to be incapable of exact interpretation.

No definite relation of micro-organisms to either wind direction or velocity is apparent. It had been hoped that this compilation would show more definite relations between some of the factors concerned. Some idea may be gained, however, of the changes in direction and velocity of the wind currents in the upper air to which floating micro-organisms must be subject.

The temperatures are such at the higher altitudes that it would seem that only those organisms capable of sustaining themselves in very severe temperatures could exist.

TABLE VI.

MICRO-ORGANISMS AND ATMOSPHERIC CONDITIONS.

Flight	Collection time (min.)	Total Bact.	Total molds	Temperature C.°		Weather Observations at time of flight	Precipitation		Wind direction	Altitude in meters	Wind velocity meters/sec.
				Ceiling	Ground		Day of Flight	Previous Day			
1	50	30	12	−21.5	2.6	Partly cloudy		2R	WNW	500	12
									WNW	0	11
2	30	32	26	−26.5	4.5	Heavy clouding overcast			W	1500	27
									WNW	500	31
									W	0	9
3		19	252	−22.5	1.0	Slight clouding on horizon			WNW	2100	20
									NW	1000	14
									W	0	3
4	50	6	6	−31.5	2.8	Clear			WNW	1500	28
									W	500	13
									W	0	4
5	50	5	13	−22	−2	Clear			W	4500	18
									WSW	2000	7
									NNE	0	2
6	60	19	12	−18	−2	Partly cloudy			N	1500	15
									N	500	19
									NW	0	10
7	60	5	5	−28	−1.5	Partly cloudy			NW	1000	4
									NNW	500	5
									NNW	0	4

TABLE VI—cont.

Flight	Collection time (min.)	Total Bact.	Total molds	Temperature C.°		Weather Observations at time of flight	Precipitation		Wind direction	Altitude in meters	Wind velocity meters/sec.
				Ceiling	Ground		Day of Flight	Previous day			
8	60	11	12	−22	0	Clear			WNW	2400	17
									NW	1500	9
									W	0	3
9	60	2	3	−33	3	Overcast, heavy clouds		7S	NW	1200	15
									WNW	500	14
									WNW	0	7
10	60	11	2	−30	−12	Clear			NNW	1800	14
									NNW	1000	18
									NW	0	7
11	44	7	10	−17	−2	Overcast, heavy clouds			W	2500	15
									WSW	1000	14
									SSW	0	3
12		4	12	−13	13	Overcast, heavy clouds					
13	60	21	0	−22	−12	Light clouding			WNW	1400	31
									WNW	500	17
									WNW	0	12
14	83	28	0	−32	−6	Clear			NW	2500	23
									WNW	1000	13
									W	0	6
15	74	10	43	−27	2	Overcast, heavy clouds			WSW	2000	20
									WSW	1000	21
									SSW	0	5
16	45	1	1	−39	4	Light haziness		11R	WSW	3500	19
									W	1500	20
									W	0	4
17	60	1	2	−30	9	Light cloudiness		1R			
18	75	5	1	−34	4	Clear			NW	4000	26
									NW	1500	16
									W	0	4
19	100	5	0	−32	+1	Heavy clouds	1R		N	800	9
						snow	13S		NNW	500	14
									NW	0	7
20	60	3	0	−29	0	Heavy clouds		2R	NNW	1200	10
						sleet		17S	NNW	500	10
									NW	0	5
21	79	10	2	−36	−11	Clear			NW	2500	36
									NW	1000	24
									WNW	0	9
22	51	11	0	−27	−4	Clear		5R	WNW	2000	24
									NW	1000	18
									WNW	0	7
23	75	2	1	−23	3	Partly cloudy			WSW	3000	14
									W	1500	16
									SW	0	5

TABLE VI—cont.

Flight	Collection time (min.)	Total Bact.	Total molds	Temperature C.°		Weather Observations at time of flight	Precipitation		Wind direction	Altitude in meters	Wind velocity meters/sec.
				Ceiling	Ground		Day of Flight	Previous day			
24	75	4	0	−25	4	Partly cloudy			WNW	2500	27
									NW	1000	21
									WNW	0	4
25	75	6	0	−38	3	Partly cloudy	3R		N	2500	19
							1S		NNE	1500	17
									NNW	0	7
26	60	1	0	−31	5	Clear			NNW	3000	22
									NNW	1500	22
									NW	0	6
27	100	21	3	−10	9	Overcast, rain	8R		WNW	5000	18
									NE	500	5
									NNW	0	1
28	100	1	1	−17	13	Cloudy	3M	13M			
29	78	4	0	−20	9	Clear, cloudy above ceiling			NE	5500	4
									ENE	1500	10
									W	0	2
30	48	4	1	−25	2	Clear					
31	68	31	24	−21	14	Overcast, snow near ceiling	3R		W	3500	15
									WSW	1500	14
									SW	0	4
32	76	2	0	−25	7	Clear			WNW	4400	28
									W	2000	18
									WSW	0	3
33	60	1	1	−11	17	Cloudy					
34	73	4	3	−19	10	Clear			WNW	5000	16
									NW	2000	10
									N	0	1
35	69	8	0	−16	14	Clear		5M			
36	165	88	15			Clear					
37	45	3	6	−1	17	Overcast, rain					
38	100	4	0	−7	14	Hazy			SSE	500	3
									SSE	200	4
									SE	0	2
39	60	9	0								
40	39	1	0								

S = snow for 1 hour.
R = rain for 1 hour.
M = mist for 1 hour.

Lower Temperature and its Relation to Isolated Organisms.

One hundred and five pure cultures of bacteria which had been isolated from air samples taken at altitudes greater than 9,000 feet

were used for an experiment which is informative regarding the resistance of some of these organisms to low temperatures.

Transfers were made to fresh nutrient agar slants, after which the transfers were immediately placed in a mechanical refrigerator at 0° C. and left there for 49 days.

At this time, those cultures which showed any growth while incubated at 0° C. were placed in a tight metal container and refrigerated in an insulated box containing carbon dioxide snow. The temperature of this box was − 26° C. when the cultures were put in and after a forty-eight hour period they were taken out. At this time the temperature of the box interior was − 39° C. The cultures were then incubated at 20° C. for one week.

Of the 105 cultures, 36 were able to *grow* at 0° C. and of these 36, there were 31 which were able to grow after 48 hours at a temperature lower than − 26° C.

A similar experiment with a Torula culture, having characteristics similar to *T. niger*, showed that organism also was able to survive similar low temperatures and grow well at ordinary refrigerator temperatures.

Pollen.

During the flight collections there have been collected eight different forms, Fig. 6, which appeared to be pollen grains and these have been observed and measured. Unfortunately, it was impossible to preserve these specimens because the paper filters were later shaken in distilled water previous to plating out for micro-organisms.

On Flight 8, one specimen (A) was obtained between 11,000–14,000 feet in altitude. A few days later, on Flight 9, another specimen of the same appearance was obtained between 3,000–9,500 feet.

The examination of filters from Flight 14 showed the presence of a pollen grain (B) in the air between 14,700–17,500 feet. A different type (C) was trapped between 17,600 and 18,800 feet on the same flight.

Flight 21 was the source of one pollen grain (E), at an exposure which lasted from an altitude of 16,000 to 17,300 feet.

The specimens previous to those obtained in Flight 36 were found singly. On this flight, which took place at relatively low altitudes, (500 to 2000 feet) there were 102 pollen grains trapped at the 2000 foot level, 98 at the 1000 foot level and 172 at the 500 foot level. The same pollen (F) was found in small numbers on Flight 37.

In addition, two different types of pollen were found on Flight 37,

one (G) being taken in the sample below 7,600 feet, and a second (H) in the exposure between 7,800 to 12,400 feet.

Several difficulties prevent the definite identification of these pollen grains. One of these is the fact that pollen grains are likely to alter their appearance under different conditions of humidity. In this

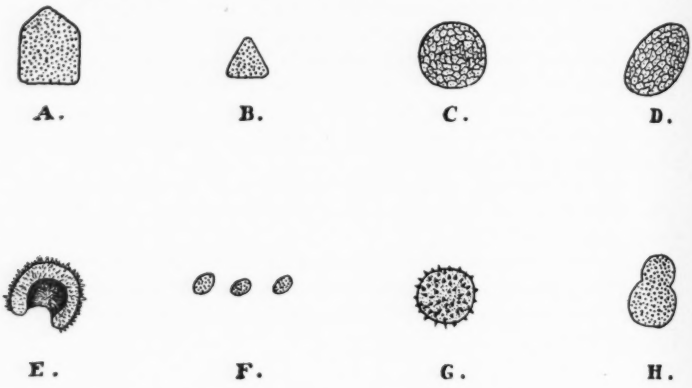


FIGURE 6. Pollen forms observed in upper air collections.

Form	Source Flight No.	Altitude (feet)	Size μ
A	8	11,000–14,000	107
	9	3,000–9,500	
B	14	14,700–17,500	72
C	14	17,600–18,700	118
D		11,500–ground	125
E	21	16,000–17,300	88
F	36	2,000	39
G	37	7,600–500	73
H	37	7,800–12,400	52

investigation there was no way of determining the humidity. Neither could the mounting of the pollens be changed to observe them in different positions, which would have been most helpful. Any change in position, mounting, or humidity was impossible because of the subsequent procedures which were undertaken for other purposes.

No attempt has been made to classify these pollens. An examination of the literature suggests the possibility that some of those found

may be members of the Pinaceae, (conifers) Caprifoliaceae, (honeysuckles) or the Ranunculaceae, the buttercups, but due to the factors mentioned above, any definite conclusions seem unfounded.

It is very interesting, however, to note that these pollens have been obtained at heights of over 16,000 feet on at least two occasions and several times at over 10,000 feet.

These observations indicate the wide areas over which plants which produce wind-borne pollens may be pollinated.

Dust Counts and Their Relation to Micro-organism Counts.

The disk collectors mentioned above were examined microscopically for dust particles in the same glass cell for pollen studies. These examinations, conducted on the paper exposure disks used in 17 flights, were carried out by means of a microscope with a mechanical stage. The dust counts included the enumeration of all observed dust particles.

As one disk was always used as a control during the collections, the control disk was counted also. In spite of the fact that these disks were washed in chloroform before using, there were still some particles on the disks previous to exposure. The average number of dust particles observed in the control disks was therefore assumed to represent the number of particles on each disk and therefore subtracted from the count obtained. Micro-organism counts were carried out on the same exposures.

The following results were obtained from the dust counts on 17 flights:

Average actual dust count per disk	323.2
Average dust particles per control disk	95.4
Average dust particles per exposure	227.8
Average bacteria count, same exposures	1.53
Average mold count, same exposures	.58

As the mold and bacteria counts were made from the very same disks on which the microscopic dust counts were made, the time element and altitude in each was identical.

This indicates a ratio of micro-organisms (bacteria and molds) of 2.11 to 227.8 dust particles, or 1 micro-organism to 107.95 dust particles.

The same procedure was followed using the data obtained only in the exposures which were made entirely above 9,000 feet. The results obtained in this manner are shown below:

Average dust particle count per disk	266.2
Average dust particles per control disk	95.4
Average dust particles per exposure above 9,000 ft.	170.8
Average bacteria count, same exposures	1.25
Average mold count, same exposures	.20

In this series a ratio of 1 micro-organism to 117.93 dust particles was obtained.

The large number of dust particles to living micro-organisms is particularly interesting. In these flights, taken in part of a busy industrial region it is not entirely surprising to note a considerable quantity of dust. Doubtless part of it comes from the smoke stacks which emit vast clouds of dust at various intervals. Possibly part of it originates from the soil wended upward by convection currents. It is not without the realms of possibility that a part of this dust is of meteoric origin. One other possible source suggested itself, namely, the presence of exhaust dust from the engine of the plane.

The latter was ruled out by an experiment whereby ammonia and hydrochloric acid were blown through two separate glass tubes joined together at the end of the motor exhaust, which is under the plane. The cloud of ammonium chloride which formed, showed that the flue gases are not conducted near the inlet tube of the micro-collector, which is on the roof of the plane.

The seeming low ratio of micro-organisms to dust may also be partly due to the actinic exposure of the sun which may be lethal to certain micro-organisms. In the opinion of some meteorologists it may require several days during the winter season for air masses to attain an altitude of eighteen thousand feet. If such is the case it is conceivable that the sun's rays might cut down the numbers of micro-organisms. The earlier mentioned preponderance of spore-forming rods in the air samples is also suggestive of this possibility.

A continuation of these investigations of dust in the air, together with the collection of such information as can be obtained regarding living micro-organisms is now under way.

Discussion.

The results obtained from these micro-biological collections of the upper air suggest certain observations.

The biological population of the upper air is probably an ever-changing one, never constant either in type or distribution, due to the many factors which may interact at the same time. These factors include temperature, humidity, wind direction and velocity, precipita-

tion, contamination from the earth's surface, seasonal growth of certain plants, and doubtless others which are less obvious.

Such results as have been obtained in this work suggest that bacteria, molds, and pollens may be expected at any height included in these experiments, namely, up to 20,000 feet.

A study of the types of micro-organisms found indicate the preponderance of soil types, as might well be expected. Among the bacteria, the Bacillaceae were found particularly prevalent, with two species, *B. cereus*, and *B. subtilis* outstanding. Each of these species is a spore-former and relatively resistant to unfavorable conditions. The two outstanding genera of molds identified were Aspergillus and Penicillium, and these types are also among the more resistant to unfavorable conditions.

The height which is reached by some of these forms induces speculation regarding the horizontal movement of some of these microscopic forms. If they can go up three and four miles in the air it would seem likely that their travels in a horizontal plane might be almost limitless. Such considerations make it very easy to understand how plant diseases may be transmitted through the travels of spores.

The findings of possible biologic strata, as is suggested in Flight 21, may be the result of the moving in of an air mass at high altitude from a considerable distance. As just a few days previous to this flight there had been heavy snows in this area, the possibility of surface organisms being the organisms found is somewhat remote. It is not considered that the findings on this flight warrant such a definite conclusion, but they are quite suggestive.

The dust collections suggest further studies to determine the comparable numbers of dust particles away from the gross contamination of the metropolitan areas. Some suggestion of a decrease of these particles at higher levels has been noted. The ratio of dust particles to living organisms may be of some significance, in that if one found an extremely high ratio it might indicate something of the history of the air mass, either that the dust had long been exposed to actinic rays or that its origin was from some industrial area.

These studies indicate that we have a sheath of air around us, containing irregularly dispersed living micro-organisms, which is in a constant state of flux due to conditions inherent to the atmosphere. The types present include bacteria, molds, yeast, and actinomyces. In this same air we find dust and other suspended materials such as pollen which are subject to these same ever-changing conditions. The results obtained suggest that further and more extended investigations

of a similar nature might yield helpful information regarding the various factors which bring about the changes.

Summary.

A report was made on the findings obtained from 45 airplane flights during which 201 separate collections of micro-organisms at various levels in the upper air, up to 20,600 feet, were made. Bacteria and molds were found above 19,600 feet. Yeasts and pollens were found above 16,000 feet. Spore forming bacteria were the predominant micro-organisms. Twenty-nine species of bacteria were identified. Aspergilli and Penicillia were the predominating molds found. Dust counts were made on 17 flights and evidence was presented that these counts decrease somewhat at the higher levels.

The results of an experiment conducted to demonstrate the resistance of certain isolated bacteria to cold were included.

Acknowledgements.

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An acknowledgement should also be made to the Rockefeller Foundation for grants of financial assistance which enabled the plane to be operated on its normal mission. Especial thanks are due the writer's assistant, Mr. J. T. R. Nickerson, for his efficient service in conducting a considerable part of the laboratory work. To Dr. R. Schuler of the Stanco Laboratory in Elizabeth, N. J., thanks are due for the low freezing hydro-carbon oil furnished for the collector.

Note: The sad and untimely death by accident of Lieutenant Henry B. Harris on June 15, 1934, while participating in other activities pertaining to aviation, has removed a faithful and efficient colleague. His coöperation and personal effort in making many of the biological collections, often under difficult circumstances, in addition to his manifold duties as meteorological observer and pilot, have been indispensable in this investigation.